III. 2 a Diseases of the Respiratory Tract:

(Metaplasiae, Tumors, Inflammations etc.)

Spontaneous tumors of the respiratory tract are extremely rare in the goldhamster as emphasized already earlier at symposia (Perugia - I966, Gatlinburg - I969) by numerous researchers.

We (DONTENWILL and WIEBECKE, I966) had formerly assumed on the basis of observations on a large stock of animals that the frequency of so-called spontaneous lung adenomatoses amounts to about I/I000. FORTNER (I958) saw among 620 hamsters 3 bronchus carcinomas, KIRKMAN (I962) among 7.200 hamsters 3 adenomatoses, 2 adenomas, 2 adenocarcinomas, DUNHAM and HERROLD (I962) among 360 hamsters I adenocarcinoma, LEE and Assoc. (I963) among 37 hamsters I adenomatosis. CHESTERMAN (I964) describes the observation of benign tumors of the lung (quantity not stated). From the descriptions on hand it is not definitely recognizable whether the described adenomatoses and adenocarcinomas of the hamster lung are cases of a pseudoadenomatosis which we (CHEVALIER and DONTENWILL, I97I) count among the "adenomatoid lesions" and separate from the tumors.

While spontaneous tumors of the larynx have so far not been described and spontaneous tumors of the lung are extremely rare, many authors have reported about induced tumors of the respiratory tract. In the beginning we reported on observations of tumors of the respiratory tract after intratracheal application of Benzpyrene and after varying treatment with Nitrosamins. In addition it should be mentioned that DELLA PORTA and Assoc. (I958) and GROSS and Assoc. (I965) succeeded in producing carcinomas of the larynx, the trachea and the lung through multiple intratracheal application of DMBA. According to the experiences which we made with the examinations of the deposit of radioactively marked smoke components (DONTENWILL and Assoc. I97I), we had to count the nasal respiratory ducts as well as the oral respiratory ducts among the respiratory tract since both ducts , even if to a varying degree, can be used in the process of inhalation.

Inflammable changes occurring in the area of the oral cavity were relatively frequently provable in smoke-exposed as well as control animals. In comparison between smoke-exposed animals and controls (3 and 8) a significant influence of the smoke-treatment could not be established (chart 20) ($X^2 = 9.67$ with 4 latitudinal degrees;* ($p \approx 5\ \%$)). Inflammable changes in the area of the nasal cavity were more frequently provable in individual groups among the smoke-exposed animals, particularly among

*latitudinal degrees = Freiheitsgrade, abrev. FG maintained throughout the text. Tr. note.

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the male animals, (e.g. group 5, 6 and I4), (chart 20). In the males we also found highly significant differences between the smoke-exposed groups (particularly group 6) and the control and placebo group ($X^2 = 18.2$; FG = 4, (P<I %).

Unequivocally atypical epithelium metaplasiae of the mucous membrane of the mouth were provable neither in the control animals nor also in the smoke-exposed animals, except for one animal treated with the gas-phase of the smoke. Papillary tumors starting from the pavement epithelium of the mucous membranes of the mouth, we were able to observe only in one each of the animals treated with DMBA of group I and 2 as well as in one animal treated with DENA of group I8A. Papillary tumors in the area of the palate were found predominantly in the animals treated with DMBA, occasionally also in untreated or smoke-exposed animals (ill. 4). There were also sporadic appearances of infiltratively growing carcinomas of the oral cavity, to some extent starting from the cheek-pouch (ill. 5 and 6). A correlation to the smoke-treatment is not present.

Charts 2I and 22 show the distribution of frequency in the individual groups.

In the nasal cavity no unequivocally metaplastic changes of the mucous membrane were found as we have described them formerly after smoke-treatment (DONTENWILL 1970).

The inflammable changes of the nasal cavity occurred with approximately the same frequency in treated and untreated animals. They were alwayd accompanying symptoms of a phlegmonous change in the soft parts of the head. We calculated the frequency differences between the groups 3, 4, 5, 6 and 8 (chart 20). The differences between this frequency were examined with an X^2- Test. The test yielded for the females no significant differences ($X^2 = 4.86$ with 4 FG; (P> 5 %)). In contrast, the differences in the males were highly significant ($X^2 = 18.17$ with 4 FG; (P< I %)). These high differences in the males are exclusively attributable to the high value in group 5,

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in which I5 animals, that is I8.75 % showed inflammations of the nasal cavity. Since however in group 6 with the highest smoke dose only 5 animals, that is 6.25 %, showed inflammations of the nasal cavity, it is questionable , whether the high share in group 6 may be attributed to an effect of the smoke-treatment.

In individual animals we observed adenomas in the area of the nasal cavities which apparently proceeded from the mucus glands. It was a case of tubular adenomas which do not show an infiltrative growth process, (chart 2I and Ill. 7). Since the adenomas also appear in the control groups K and 3, a correlation to the treatment is not assumed. Malignant tumors of the nasal cavity were not observed.
(Two and one half lines illegible in German Text. Tr.n.)

... difference between males and females is 895.0 with I latitudinal degree. This corresponds to an error probability of considerably less than I %. In the females the X^2-value for the difference between the groups 3, 4, 5 and 6 amounts to I6.9 with 3 latitudinal degrees. This difference is significant at $P < I$ %. The frequency of the concrements is significantly higher in the control group 3 with 56 % than in the smoke-exposed groups 4, 5 and 6 with maximally 4I % in group 4. Consequently, the frequency of the concrements is reduced by the smoke-treatment. These generally layered stones fill the gland clearances in parts completely and lead to the atrophy of the epithelium. They are predominantly provable in female animals. No unequivocal statements can be made on the make-up of the red-brown concrements on account of the colorations which were undertaken (HE, iron, (Berlin blue) Ziehl-Neelsen, Kossa, PAS, bleaching with H_2O_2).

In individual animals we found benign tubular to papillary adenomas of Harder's Gland (chart 23 and ill. 9).

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In the pharynx papillary tumors proceeding from the pavement epithelium were provable predominantly in the animals pretreated with DMBA (chart 24), except for one beginning carcinoma in one animal of group I (ill. II)they did not show any infiltrative growth. They were only sporadically observed in untreated and smoke-exposed animals. A correlation to the smoke-treatment is not present.

Papillary tumors of the trachea (chart 24) as DONTENWILL and MOHR (I96I c), HERROLD and DUNHAM (I962, I963), MONTESANO, SAFFIOTTI and SHUBIK (I970), ALTHOFF, WILSON and MOHR (I97I) and others described them predominantly after treatment with diethylnitrosamin (DENA), we found relatively frequently in the animals treated with DENA of the groups I8 and I8A, but also sporadically in the animals treated with DMBA of t groups I and 2. The structure of papillary not infiltratively growing tumors induced through DENA treatment ressembles the morbid growths formerly described by us and compared to the pavement epithellium ressembling tumors of the bladder (ill. I2). After the more recent examinations on semi-thin profiles by ALTHOFF, WILSON and MOHR (I97I) it can be assumed that these papillary growths of the trachea are morbid growths which are not made up of metaplastic pavement epithelia but of cilia-carrying columnar epithelia, respectively of mucus producing cells. The contrasting of the two groups I and 2 as well as I8 and I8A shows that the smoke-treatment in the animals pretreated with nitrosamin has led to an increase of the papillary tracheal tumors. Since no tracheal tumors occurred through smoke-treatment alone even in high dosage, the effect visible here is to be essentially understood as a promoter effect of the smoke respectively a cocarcinogenic effect.

As already in our preliminary examinations (DONTENWILL, I970), the larynx region of the hamster shows also in the present experiment in all treated groups the most severe changes. As earlier, we divided the changes into 6 stages as they are presented schematically in ill. I3,and in the classification we have used that scheme (chart 25)

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as the basis for the division of stages. The "Atlas of Tumor Pathology" published by Ash and Assoc. (1964) served as the basis for this division of stages.

The individual stages (see ill. I3, I4 a-m) were based on the following criteria (chart 26) as characteristics. The frequency of the described larynx changes is compiled in chart 27. They shall be discussed in detail subsequently to the description of morphologically deviating findings in the larynx.

Besides these characteristic changes a papillary tumor was found in one instance in the larynx under the pattern of a fibroepithelioma in group I0 with considerably stronger development of the stroma than in the typical papillomas.

Of the described larynx changes (stage I - 6), morphologically well delimitable were papillomas which were traceable in the lower sections of the larynx only in the animals treated with DENA (group I8 and I8A). In group I8 two females and I9 males and in group I8A 5 females and I0 males had larynx papillomas. The sex difference is significant.

These papillomas showed in their structure a vast similarily to the papillomas of the trachea observed after nitrosamin treatment, in respect to the tissue structure as well as the total structure. (ill. I5 a and b).

In the comparative observation of the larynx changes we settled first of all the question whether leukoplakias of the larynx respectively larynx tumors were traceable in the altogether 960 not-treated animals (which means, not smoke-exposed and not treated with carcinogens). We found only in one animal of group K and in no animal of the groups 3 and 8 changes in the sense of stage 2, in none of the animals the stages 3 - 6. Only stage I was traceable, to some extent combined with inflammable processes. Also in the animals of group 7 treated only with the gas-phase, only

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pachydermias (stage I) were found in 9 animals, which means, no larynx changes of stages 2 - 6. Larynx changes of the stages 2 - 6 were also not observed in the animals of group I3 and group I8A. In the evaluation of the different effect of the treatment within the individual groups in comparison to the controls, we included in our calculation the time of the appearance of the changes in the larynx as well as the degree of severity of the changes. The frequency distribution of the stages 2 - 6 was evaluated in the same way as the age of the animals at the time of the examination respectively at the time of the proof of the described larynx changes (chart 27 - 37). In viewing the charts, it must be taken into consideration that often several stages appeared side by side respectively simultaneously in the same animal.

Correlation between Age (Survival Period) and Stage of the Leukoplakia of the Larynx

In order to be able to analyse this correlation, we counted for each treatment group separated in male and female, the frequency of the different leukoplakia stages in 5 different age groups (0-25, 26-50, 51-75, 76-100, >100 weeks). The results are compiled in the charts 28 - 37. It is to be observed thereby that one animal could show several stages simultaneously. The total number of stages can therefore be higher than the number of animals within a certain age group. The percentages listed with the discovered numbers relate in each instance to the number of animals within the age group in question. This number is listed in the second column.

The charts show that in all smoke-exposed groups, with exception of group 7 treated with the gas-phase of the smoke, there exists a pronounced dependency between the age and the leukoplakia stage; high leukoplakia stages occur predominantly with advanced age; that is, all the animals of this age group often have one leukoplakia within stage 3 - 5 respectively carcinomas (stage 6). For the quantification of these dependencies, Pearson's Contingency Measure was calculated for the mentioned

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groups. The males and females were thereby taken together in one group eventhough different age distributions do indeed exist in the sexes, but the dependency ratios are not influenced by them. These contingency tables and the appertaining contingency measures are listed in charts 38 - 42. These contingency measures show a high dependency between age and leukoplakia stage.

It must be observed that these contingency tables are relative to the distribution of leukoplakia cases and not the number of animals. Accordingly, the total number is not a constant quantity but a coincidental quantity. Moreover, the numbers in the different stages are statistically dependent since to some extent they refer to the same animals. The quantity X^2 is therefore not X^2 distributed.

In order to test the correlation between age groups and leukoplakia stages, ARMITAGE (I97I), suggested to compare the frequency of the stages with a Poisson distribution whose parameter is estimated from all age distributions. This test resulted in all contingency tables in a highly significant dependency.

It is remarkable that the distribution of leukoplakia stages can be divided into three different types.

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The first type is present in the smoke-exposed groups 5 and 6. In this type the stages 3 and 5 occur with approximately the same frequency, and approximately 25 - 30 % of the animals are afflicted by it. The age distribution of stage 5 is shifted more to the higher age groups in comparison to the distribution of stage 3.

The second type is present in the groups 4, 9, I0 and II. In this type stage 3 predominates but it also includes only 25 - 30 % of the animals. The high stages, particularly stage 5 and 6 are relatively rare.

The third type is present in the groups I2 and I4 - I8. It is characterized by the great frequency of stage 3 (65 - 78 %) which occurs predominantly in the age groups 5I - 75 weeks. It is also striking in this distribution that stage 5 occurs relatively frequently and stage 4 relatively rarely. In these groups stage 4 often seems to be

Source: https://www.industrydocuments.ucsf.edu/docs/nlkk0000

"skipped". This is corroborated by the fact that the age distribution of stages 3 and 5 runs similarly while the distribution of stage 4 is shifted somewhat more to the younger age groups.

The dose/effect correlation is particularly unequivocal in the comparison of groups I, 2, 4, 5, 6 with the controls (groups 3, 8 and K). While the controls showed almost no larynx changes of the stages 2 - 6 (only one case of stage 2 in group K), the groups 2, 4, 5, 6, I showed in approximately this sequence changes in the area of the larynx to an increasing extent. Particularly the degree of severity (of stages 2 - 6) was thereby increasing with increased length of application and increasing dose, whereby the smoke-exposed animals with additional DMBA-treatment showed the strongest findings (group I).

Pronounced was the considerable difference in the comparison between the effect of DMBA alone as well as DMBA and smoke or smoke alone, this becomes particularly evident in the female animals and in the total number of animals.

This not simply additively explainable increase of the effect through initial treatment with DMBA, particularly in the female animals, argues for an influence of cocarcinogenic factors (besides the carcinogenic factors) of smoke.

The comparison between group 5 and the groups 9, IO and II, that is, between the same-dosed treatment with the standard cigarette and the treatment with the cigarettes containing natrium-nitrate, the sheet containing natrium-nitrate and the sheet alone demonstrates that particularly the larynx changes stage 5 and 6 are reduced in the groups 9, IO and II. This reduction cannot be explained through a varying treatment time. The natrium-nitrate admixture to the tobacco produces therefore, just as in the skin-test (DONTENWILL and others, I972) a strong reduction of the biological effect of the smoke. The fact that, with additional nitrosamin treatment, not an increased but indeed a reduced effect is provable, argues against an additive effect of smoke

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and nitrosamin on the same place in the larynx. Nitrosamin alone is not able in the applied dose to produce morphologically equivalent larynx changes of the stages 2 - 6, as they occur in smoke-exposed animals.

The cigarettes manufctured from tobacco sheets show - similar to the skin-test (DONTENWILL and others, I972) - a distinctly reduced biological effect. In both tests (cigarettes with natrium-nitrate and cigarettes from sheets) an effect is provable which runs approximately parallel to that of the skin-test.

The minor lung changes in group I3 (preliminary treatment with asbestos) show that the asbestos dose which was used, was too low. The comparison between group 5 and I2 shows that almost the same result could be obtained with the same smoke dose in the animals which were taken into the test 6 months later.

A considerable reduction of the biological activity, measured by the frequency of the stages 5 and 6, was also shown by the groups I4 (black cigarette), I5 (cigarette with acetate filter) and I6 (cigarette with Dico-filter). The reduction is somewhat smaller for the cigarette with charcoal filter. The very frequent appearance of stage 3 is conspicuous in these groups. Approximately 70 % of the animals of these groups showed larynx changes in the sense of stage 3.

The fact that in group 7 (treatment with the gas-phase of the smoke) changes of the stages 2 - 6 did not occur, allows us to assume with certainty that the carcinogenic factors are to be sought chiefly in the particle-phase.

In viewing the charts, it becomes furthermore evident , that with a relatively weak influence respectively at the beginning of the treatment, stage 3 predominates in the larynx changes, and that with an increasing degree of severity of the larynx changes and an increasing length of the treatment, stage 3 of the larynx changes decreases while in contrast the stages 4 - 6 increase at the same time. Consequently, it is a case of a, so to speak, step by step malignization of the epithelium whereby

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it is not necessary that all stages have to be passed through.

The lacking infiltration into the cartiladge of epithelium growths in stage 6, designated as "early invasive carcinoma", is a finding which can be explained by the reaction between tumor growth and cartiladge. Also in earlier examinations (DONTENWILL and MOHR, I96I), we did not see any carcinoma infiltration into the cartiladges of the trachea or the larynx.

The tumor growth spreads almost exclusively between the cartiladge links respectively in the zone of the pars membranacea. In comparative examinations on the ears of mice (DONTENWILL and Associates I972), we were able to show that also in the large tumors of the mouse-ear, produced by Benzpyrene, the cartiladge is almost never attacked by the tumor growth. Apparently, the cartiladge constitutes an extraordinarily resistant barrier against induced tumor growth.

A joint quantitative evaluation of the individual stages of the larynx changes and with that a summarization of these changes per group does not seem to be possible. The present examinations demonstrate solely that stage 3 apparently constitutes a first step towards the stages 5 and 6 and that the stages 5 and 6 are expressions of a stronger tumor-triggering effect. While in our opinion, in comparison to the experiences in man (BUNGELER and DONTENWILL, I954, DONTENWILL, I966), the stages 2, 3 and 4 can be assessed as precanceroses "in the wider meaning", we would like to regard stage 5 as a precancerosis "in the narrower meaning".

Inflammable changes of the larynx were not unequivocally more frequently provable in the smoke-exposed animals as compared to the controls (chart 20) (X^2 = I3.04 with 4 FG (P< I %)). Inflammations and tumor formation show no parallelity.

In relation to the changes observed in the upper respiratory tract area, the number of tumors of the bronchi and the lung are extraordinarily small. Bronchial papillomas (chart 24) as they had been described formerly with nitrosamin treatment and the

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influence of Benzpyrene, we found only sporadically in the animals treated solely with DMBA and in the hamsters of group I8 and I8A treated with nitrosamin (ill. I6 a plus b). The varying localization of DMBA - respectively DENA - induced changes is thereby remarkable as was already found in the comparison of the place of attack of smoke-components and nitrosamin.

In the lung we could prove only one infiltratively growing pavement epithelium carcinoma in one animal treated simultaneously with smoke and DMBA (ill. I7).

In 3 animals (2 males of group 2 and I female of group 5) we saw mucus-producing adenomas (ill. I8) proceeding from the bronchus, as SAFFIOTTI (I970) described them after intratracheal BP-application.

Unequivocal metaplasiae of the bronchial mucous membrane were found relatively rarely. Metaplasiae were found only in group I (2 animals) and 6 (I animal), that is, in animals which were additionally treated with DMBA or smoke-treated with the highest dose. Bronchial epithelium changes,as AUERBACH (I97I) was able to prove them in the chronic smoke-treatment of dogs,were not observed by us.

Lung: Macrophagi, Microliths, "Adenomatoid Lesions".

Of importance is also the frequency distribution of the following lung changes:

I. Macrophagi accumulations in the alveoli with brown, iron-positive pigment which we registered after OTTO (I963) as "smoke cells".
2. Accumulations of pigment-free macrophagi,
3. The appearance of microliths in the alveoli,
4. The frequency of "adenomatoid lesions" of the lung.

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Chart 43 shows that the so-called "smoke cells" (ill. I9) also occur in not smoke-exposed animals and are therefore not to be understood as specific changes, that is, are not actually to be understood as "smoke-cells in the meaning of OTTO, which

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has already been pointed out by CONNING (1969). The frequency of the "smoke cells" is unequivocally higher in the smoke-exposed groups than in the control groups K, 3 and 8 ($X^2 = 176.5$ with 6 FG ($P < 1$ %)) in the females and ($X^2 = 141.3$ with 6 FG ($P < 1$ %) in the males. It can be assumed that the particle-phase of the smoke is more strongly responsible for that than the gas-phase which becomes particularly evident in the male hamsters in the comparison of the groups 5 and 6 to 7 ($X^2 = 38.6$ with 2 FG ($P < 1$ The finding for group I (DMBA and smoke) and I2 (asbestos and smoke) shows that other factors can also influence the frequency of the "smoke cells"(differences between the groups I, I2 and 3 highly significant ($X^2 = 144.8$ with 2 FG ($P < 1$ %)).

Pigment-free macrophagi appeared particularly in the animals pretreated with asbestos of the groups I2 and I3 whereby often asbestos particles (ill. 20) and ironpigment deposits became visible.

The distinct increase of pigment-free macrophagi is also found in the animals of group I2 pretreated with asbestos and smoke.

The microliths show the greatest frequency apparently in the animals pretreated with DMBA. The difference to group K is highly significant ($X^2 = 81.4$ with 2 FG). On the other hand,there is no significant difference between the smoke-exposed groups 4, 5 and 6 and control group 3 as well as placebo group 8 ($X^2 = 4.02$ with 4 FG). It is possible that microliths are formed after cell damages through the intratracheally applied cancerogen, solved in CMC,which was the cause of microliths formation.

"Adenomatoid Lesions" of the Lung

In chart 43 we compiled the changes in the sense of a pseudoadenomatosis, better the so-called "adenomatoid lesions"which were traceable in the individual groups.

The pseudoadenomatoses or "adenomatoid lesions" belong to the most frequent lung changes in the hamster colony which we used for the present experiment and show a very varied range. They are, as we had already earlier mentioned in detail, not to be mistaken

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for carcinomas of the lung (CHEVALIER and DONTENWILL, 1971). Earlier we dealt in detail with the genesis of the "adenomatoid lesions" of the lung and were able to find that also in the present examinations, the "pseudoadenomatosis" particularly with a simultaneous appearance of inflammable lung changes (bronchopneumonias) was often very strong in its range. Ill. 22 a and b show one diffuse and one focal "pseudoadenomatosis" whereby the cilia-carrying epithelia of the bronchus almost completely line the alveoli and thereby give the misleading impression of adenoma-ressembling changes which are often falsely interpreted as tumors (see CHEVALIER and DONTENWILL, 1971). "Adenomatoid lesions" respectively pseudoadenomatoses were also traceable in the controls as chart 43 indicates. An influence of the treatment on their frequency is statistically only weakly ascertained ($X^2 = 11.67$ with 4 DFG ($P > 1\ \%$)). A dose dependency is however not provable ($P > 5\ \%$).

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Lung: Thrombi - Infarcts - Vascular Wall Inflammations

After chronic smoke-treatment of dogs HAMMOND, AUERBACH, KIRMAN and GARFINKEL (1970) and others, observed an increased appearance of lung thrombi.

We saw in the lung thrombi as well as inflammable vascular changes often combined with lung vessel thrombi in the sense of a thrombangiitis. Dense inflammable infiltrates (particularly leukocytes and lymphocytes) in the vessel wall and in the vessel vicinity and vascular wall swelling to the point of fibrinoid necrosis were traceable thereby. The findings show a vast similarity to inflammable vascular changes of the coronary arteries and the peripheral arteries.

Chart x plus y offers a survey of the frequency of thrombi and inflammable vascular processes. A hemorrhagic infarct of the lung was observed only in one animal of group

The comparison of the frequencies between the individual groups does not show an increase of the changes in the smoke-exposed animals. In the females the test also yielded differences between the groups 3, 4, 5, 6 and 8, an χ^2 of 4.52 and in the males of 2.44, for 4 latitudinal degrees in each instance. These differences are not significant ($P > 5\ \%$).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlkk0000

Lung Emphysema - Pneumonia - Bronchitis

HALMOND and Assoc.(1970) as well as AUERBACH and Assoc. (1970) reported one lung emphysema in the dog after chronic smoke-treatment. Also in other smoke-treatment experiments, for instance, in the rabbit (HOLLAND and Assoc. 1963) the formation of a lung emphysema was reported.

In the hamster, an extraordinarily strong lung emphysema after treatment with papain, was described by several authors (GOLDRING et al. 1968, GOLDRING and Assoc. 1970, GROSS and Assoc. 1968, 1971). The lung emphysema proven in these experiments was as a rule traceable throughout the entire lung and often showed an extreme expansion. In these changes the lung emphysema could be diagnosed as a rule already in simple histological examinations.

Through numerous examinations on man it was possible to demonstrate that the diagnosis lung emphysema can only be ascertained with specific methods on tissue profiles (HARTUNG 1959; OTTO 1968a, b).

As we already mentioned in the beginning, we fixed the lungs in our experiments after the method described by CHEVALIER (1971), in order to be able to proceed from a dilated state of the lung in vivo in the comparative evaluation of the lung changes and particularly in the measuring of the degree of severity of the lung emphysema. All lungs were largely fixed in the same state of dilation which facilitated a comparative evaluation of the condition of the lung alveoli. As the chart shows, an unequivocal emphysema according to criteria used in human pathology could be diagnosed only in a few animals during the microscopic examination of the lungs.

An unequivocal accumulation of the diagnosis emphysema, ill. 23, was not recognizable in the treated animals (chart 45). Since it is not possible with the small animal to carry out the process of emphysema evaluation used today in human pathology on a paper-mounted large surface profile with the aid of a screen-system (OTTO 1970), we attempted to carry out quantitatively comparative measurements through other methods.

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We made comparative measurements on the following groups:

Group K : 10 ♂ and 10 ♀ animals with an age of 9 - 32 weeks (=4. - 27. test week) was compared to

Group 3 : 20 ♂ animals of the 104. - 129. test week and
20 ♀ of the 65. - 93. test week as well as

Group 6 : 20 ♂ animals of the 71. - 111. test week and

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20 ♀ animals of the 56. - 87. test week.

The animals of group 6 had been smoke-treated daily with 3 x 30 cigarettes. We selected these three groups in order to compare lungs of healthy, young animals (group K) with lungs of untreated controls (group 3) of a more advanced age and animals which had been smoke-treated with a high dose for a long time.

All lungs were fixed after the initially described method, which means, dilated to the normal volume and subsequently embedded in the usual manner, cut and colored. We were careful to keep a largely constant cut-width and a coloration of even strength. In order to arrive at an evaluation largely independent from the researcher in the assessment of the lungs, we decided for a densiometric process of emphysema measuring (GLAUSER and GLAUSER 1968, LONGFIELD and HENTEL 1969). The measurements were carried out with the microscope-photometer MPV of the Leitz Co. after attachment of a grey-filter.

We measured the transparency in % in a lung area of always the same size. This could be focussed precisely with the help of the available cross-wires and square diaphragms (o.6 x 0. (illegible, Tr.) mm). The examinations were carried out in a darkened room. The zero-value was re-examined before each measurement. In the focussing of the lung sections which were to be measured, we were carefull to make sure that no larger bronchi or blood-vessels rested within the measuring area. This was the reason why the measuring area could not be chosen much larger.

We made 5 respectively Io measurements per lung. Lungs with strongly inflammable changes, atelectases or strong congestion could not be applied in the evaluation. The measurements resulted in the following:

male animals:

	group K	group 3	group 6
number of animals	IO	20	20
number of measurements	IOO	IOO	IOO
transparency			
% average value	80.63	80.62	8I.29

According to the variance analysis the differences between the groups were statistically not significant (P> 5 %).

female animals:

	group K	group 3	group 6
number of animals	IO	20	20
number of measurements	IOO	IOO	IOO
transparency			
% average value	79.38	79.84	79.II

Here too no difference could be found (P> 5 %).

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Source: https://www.industrydocuments.ucsf.edu/docs/nlkb0000

The present results demonstrate that a difference between the controls and the chronically smoke-exposed animals was not determinable through a densiometric evaluation. Consequently, the lung emphysema rate was not increased by the smoke-treatment.

For the purpose of controling the densiometric evaluation we used a further method. Since, as mentioned in the beginning, an evaluation by means of large surface profiles was not possible, we tried to do quantitative measuring with a similar method.

The lungs were projected on the table top in medium enlargement (object. A 0.25) by means of a drawing-mirror (Leitz). A screen of I0 mm side length and 2 mm gradation, mounted on the ocular (I2x) made a counting of the lung alveoli possible on drawing-paper. The actual size of the measured zone, that is, the zone delimited by the screen was 0.93 x 0.93 mm. All the alveoli or small bronchi within the range of the screen and projected onto the paper were marked on the paper with an ink-pen. Five different zones were evaluated per lung. The number of the marked cavities was subsequently counted. In contrast to the densiometric method, the profiles of all lungs with two exceptions (one atelectatic lung and one lung with catarrhalic inflammation) could be evaluated.

Since differences between smoke-exposed and not smoke-exposed animals among the male animals with a longer survival period were the most likely to be expected, the lungs of group 3 and 6 were examined. This yielded no significant differences ($P > 5\ \%$).

male animals:

	group 3 controls I04. - I29. week	group 6 smoke-exposed animals 7I. - III. week
number of animals	20	20
number of measurements	I00	I00
alveoli/unit, average value	I76.27	I70.96

The present comparative examinations show that with high-dosed, long-lasting smoke-exposure, an unequivocal increase in lung emphysema in the smoke-exposed animals was not provable with the applied measuring methods.

To supplement the evaluation it must be added that stronger inflammable changes in

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

the area of the bronchi (with exception in the range of bronchopneumonias) were extraordinarily rare in the controls and the treated animals. Chart 45 shows the frequency of bronchopneumonia and of severe bronchitis in the individual test groups. A significant increase of these findings was not provable in the smoke-exposed animals. Whether the maintenance of the test animals as described in the beginning is also responsible for that can not be determined.

It should be mentioned in addition that bronchiectases with transition to the so-called cyst lung were observed only once. Whether their formation constitutes a similar process as in the cysts of the liver and other organs can not be evaluated on the one case.

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Source: https://www.industrydocuments.ucsf.edu/docs/nkk00000